Journal of Medical Virology 53:60–62 (1997)

Analysis of United Kingdom Wild-Type Strains of Varicella-Zoster Virus: Differentiation From the Oka Vaccine Strain

K. Hawrami[1,2] and J. Breuer[1,2]*

[1]*Department of Medical Microbiology, St. Bartholomew's, London, England*
[2]*Royal London School of Medicine and Dentistry, London, England*

In Japan and the United States, where vaccination against varicella-zoster virus (VZV) infection with the live attenuated Oka strain of varicella is routine, cases of chickenpox or shingles occurring in vaccinees can be caused by either wild-type or vaccine virus. Differentiating such cases is important epidemiologically and can be achieved only using molecular typing methods. In the United Kingdom, the Oka vaccine is being considered for use in groups at risk of severe primary varicella, such as seronegative immunocompromised patients and women who may be considering pregnancy. In addition, seronegative health workers who may be occupationally exposed to VZV infection might also be offered vaccination. We analysed 249 U.K. wild-type VZV strains, 105 from cases of chickenpox and 144 from shingles cases, to determine whether they could be distinguished from Oka by the genotyping systems used in Japan and the United States. Four polymorphic loci were examined, a *Pst 1* restriction site in gene 38, a *Bgl 1* restriction site in gene 54, the R5 repeat region, and the R2 repeat region. The results suggest that U.K. strains of VZV are more similar to U.S. strains than to Japanese strains. All the U.K. wild-type viruses were positive for the *Pst 1*-1 restriction site, unlike Oka, which is negative. However, one of thirty strains was indistinguishable from Oka at all other loci. ***J. Med. Virol. 53:60–62, 1997.*** © 1997 Wiley-Liss, Inc.

KEY WORDS: varicella-zoster virus; typing; molecular epidemiology; vaccine; PCR; Oka

INTRODUCTION

The live attenuated Oka vaccine against varicella was first developed in the 1970s [Takahashi et al., 1974]. Since that time it has been shown to be effective in preventing varicella infection in vaccinated leukaemic children in remission [Gershon et al., 1985], other immunocompromised groups [Prober et al., 1982; Broyer and Boudailliez, 1985], and healthy children [Takahashi et al., 1985; Weibel et al., 1984; Asano et al., 1994] and adults [Gershon et al., 1988]. Most recently the vaccine has been licensed for use in children in the United States. Although there are no plans to license the vaccine for general use in the United Kingdom, increased awareness of the problems of varicella among hospital staff [Jones and Reeves, 1997] and pregnant women [Seidman et al., 1996] has prompted consideration of a targeted vaccination program to reduce primary infection in these vulnerable groups [Friedman-Ross and Lantos, 1995].

The vaccine results in protective immunity in 90% of healthy adults [Gershon et al., 1988]. However, a small number of healthy recipients will develop vaccine chickenpox [Gershon et al., 1992; Asano et al., 1994] or subsequent breakthrough chickenpox [Asano et al., 1994] caused by circulating wild-type virus. In addition, reactivation of the vaccine strain to cause zoster occurs in up to 6% of vaccinees [Gershon et al., 1992], and reactivation of wild-type varicella-zoster virus (VZV) or a recombinant strain in a previously vaccinated individual has also been described [Shiraki 1991]. Monitoring of these and other potential vaccine-related complications, as well as transmission events, requires that the vaccine strain be differentiated from wild-type viruses. This can be done only through genetic typing, because wild-type and vaccines strains are indistinguishable in in vitro biological or animal assays. In the United States, where the vaccine is licensed, wild-type and vaccine strains can be distinguished by amplification of VZV DNA across a region in gene 38 that is polymorphic for a *Pst 1* site, followed by *Pst 1* digestion of the product [La Russa et al., 1992]. In

*Correspondence to: J. Breuer, Department of Medical Microbiology and Virology, The Medical Colleges of London and St. Bart's, Queen Mary Westfield College, 37 Ashfield St., London E1 1BB, England. E-mail: jbreuer@qmw.mds.ac.uk

Accepted 17 April 1997

TABLE I. VZV Primers

Region	Primer	5′ Primer sequence 3′	T_m (°C)	Size of PCR products (bp)
R2	R2 F	CGGTAAATCTGGCATGCG	55	42n + 478
	R2B	AAGGGGAGCGTGGATGTGTC		
R5	R5F[a]	GGCAAATACTTAGACCGTTTT	55	88n + 24n + 189
	R5B	TAATGGACTTTTAATGGATTG		
Bgl 1 gene 54	BglIF[b]	TCCCTTCATGCCCGTTACAT	52	222 (137 + 85)
	BglIB	GGAACCCCTGCACCATTAAA		
	BglI/Eng	GACCAAACACTCTgCCTTCATGgCCGT	55	
Pst 11 gene 38	Pst IF[c]	AAGTTTCAGCCAACGTGCCAATAAA	55	647 (290 + 357)
	Pst 1B	AGACGCGCTTAACGGAAGTAACG		

[a]Shoji et al., 1992.
[b]LaRussa et al., 1992.
[c]Takada et al., 1995.

Japan, a more extensive typing system is required, involving additional amplification across two polymorphic repeat regions (R5 and R2) and a *Bgl 1* restriction site in gene 54 [Takada et al., 1995]. Even then, 7% of wild-type strains are identical to Oka. We analysed 249 viruses from cases of chickenpox and zoster to determine whether the established U.S. and Japanese typing methods would distinguish U.K. strains from the Oka vaccine strain.

MATERIALS AND METHODS

One hundred forty-four viruses from cases of zoster and 105 from cases of chickenpox were typed. Most of these cases presented to three hospitals in London between 1994 and 1996. Fifty-one of the viruses came through general practitioners and infectious diseases physicians throughout the United Kingdom. Approximately 20% of the viruses were isolates, and the rest were primary virus typed directly from vesicle fluid in viral transport medium or air dried onto slides [Hawrami et al., 1996].

All 249 viruses were typed by the polymerase chain reaction, using primers across three well-described variable markers (Table I). The products from genes 38 and 54 were digested with *Pst 1* and *Bgl 1* restriction enzymes, respectively. A restriction site was engineered into the forward *Bgl 1* primer to control for the restriction analysis in *Bgl 1*-negative products (Fig. 1, lanes 9 and 10). The products from R5 were analysed for size polymorphisms by electrophoresis on 2% agarose gel [Hawrami et al., 1996]. A subset of 31 viruses was also typed using primers to the less sensitive R2 repeat region [Hawrami et al., 1996]. In this case the products were sequenced directly on an ABI 377 sequencer using dye terminators to determine the exact number of repeated units. Viruses were typed as positive or negative for *Pst 1* or *Bgl 1* sites; type A, B, or C for R5; and type $R2_7$ (i.e., Oka-like, containing seven subunit repeats) [Takada et al., 1995]; or $R2_w$ for non-Oka-like viruses, i.e., those with fewer or more subunit repeats.

RESULTS AND DISCUSSION

The results of the typing at each locus are shown in Table II. No difference was found between strains of virus causing varicella and those causing zoster. All the strains examined were, unlike Oka, positive for the *Pst 1* restriction site in gene 38. This is similar to the strains circulating in the United States [La Russa et al., 1992] and unlike the wild-type VZV strains circulating in Japan, up to 37% of which are negative for *Pst 1.* U.K. strains also resembled the U.S. wild-type viral strains, 20% being positive for the *Bgl 1* site in gene 38 [LaRussa et al., 1992]. By contrast, U.K. strains differed from Japanese strains at the R5 locus [Takada et al., 1995]. Over 95% of U.K. strains were type A and the rest type B, whereas 13% of the Japanese strains

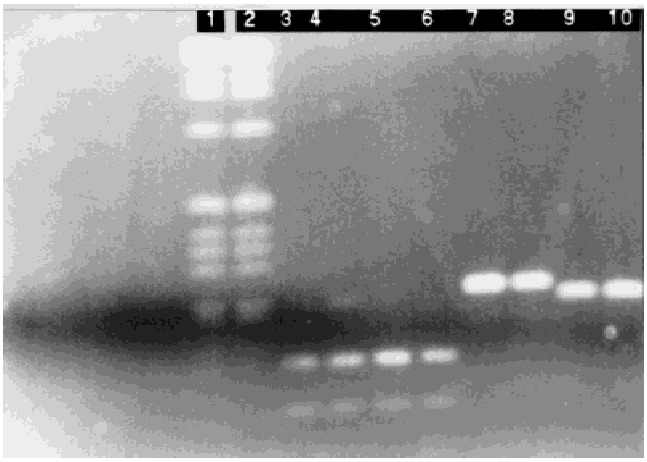

Fig. 1. Agarose gel electrophoresis to show a *Bgl 1* digest of DNA fragments amplified from varicella zoster virus gene 54.

TABLE II. Genotypes of U.K. Strains of VZV

Type (*Pst 1*, *Bgl 1*, R5)		Total n = 249 (%)	Type (R2)	Total n = 31 (%)
Oka	*Pst 1* − *Bgl 1* + R5A	0		
	Pst 1 + *Bgl 1* + R5A	46 (19)	R_7	1 (3)
			R_w	5 (17)
	Pst 1 + *Bgl 1* + R5B	3 (1)	R_7	1 (3)
			R_w	1 (3)
	Pst 1 + *Bgl 1* − R5A	191 (77)	R_7	1 (3)
			R_w	15 (48)
	Pst 1 + *Bgl 1* − R5B	7 (2.8)	R_7	3 (10)
			R_w	4 (13)

were type A, 76% type B, and 7% type C [Takada et al., 1995]. Seventy-seven percent of strains of VZV circulating in the United Kingdom were typed as *Pst 1* R5A *Bgl 1*+, with the majority of these unlike Oka at R2 (Table II). Overall, these results suggest that strains of VZV in the United Kingdom are more similar to those found in the United States than in Japan. Of the 31 viruses typed at the R2 marker, 6 (19%) were, like Oka, type $R2_7$. One of these was also R5 A, *Bgl 1*+, i.e., indistinguishable from Oka, except by the *Pst 1* site. Seven percent of wild-type Japanese viruses are completely indistinguishable from Oka in the typing system employed, being *Pst 1*−, R5A, *Bgl 1*+, and $R2_7$ [Takada et al., 1995].

In summary, U.K. wild-type strains of VZV can, at present, be distinguished from the Oka vaccine strain at the *Pst 1* locus in gene 38. However, the data suggest that viruses that are indistinguishable from Oka at other loci are circulating in the community. Identification of further polymorphic loci may become necessary for typing of vaccine and wild-type strains if *Pst 1*− strains enter the United Kingdom.

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